PERGAMON

Chemosphere 43 (2001) 355–361

CHEMOSPHERE

Determination of the levels of aromatic amines in indoor and outdoor air in Italy

G. Palmiotto [a], G. Pieraccini [b], G. Moneti [a], P. Dolara [a,*]

[a] *Department of Pharmacology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy*
[b] *Interdepartmental Center for Mass Spectrometry, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy*

Received 8 June 1999; accepted 27 March 2000

Abstract

We studied the concentration of 10 primary aromatic amines (AA), which are classified as suspected carcinogens, in indoor and outdoor air in Italy. The measured AA included: aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, 2,3-dimethylaniline, 2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, 2-naphtylamine and 4-aminobiphenyl. In the indoor environment (homes, offices and public buildings) the level of contamination (expressed as sum of 9 AA, excluding aniline) varied from 3 ng/m^3 (hospital ward) to 207 ng/m^3 (discotheque). In most indoor environments with no contamination from cigarette smoke the AA levels were below 20 ng/m^3, whereas in the presence of smokers higher values were observed. Aniline levels were more erratic (varying from 53 ng/m^3 (office of non-smokers) to 1929 ng/m^3 (discotheque) and were not related to cigarette smoke. The concentration range of AA (excluding aniline) in the outside air varied from 3 ng/m^3 (Siena) to 104 ng/m^3 (Brindisi); aniline concentration was extremely variable. Most samples of outdoor air had AA levels lower than 40 ng/m^3. In conclusion, AA are widespread air contaminants and attain a high concentration in heavily contaminated indoor environments, due to smoking and poor ventilation. AA occasionally attain a high level in outdoor air as well. Therefore, a strategy of reduction of the exposure to AA should consider the abatement of multiple sources of contamination.

Keywords: Aromatic amines; Aniline; Smoking; Air pollution

1. Introduction

Primary aromatic amines (AA) are biologically active compounds, well known as environmental pollutants. AA are common by-products of chemical manufacturing and contaminants of dyes, rubber and textiles; they can also originate from gasoline and coal combustion (IARC, 1972, 1974a,b; 1982, 1986; Humphrey et al., 1992).

Two AA (2-naphtylamine and 4-aminobiphenyl) are known to be carcinogenic for humans (IARC, 1972, 1974a,b,c). Others (*o*-, *m*-and *p*-toluidines; 2,3-, 2-4-, 2,5- and 2,6-dimethylanilines) are suspected carcinogens although their association with human tumours has never been adequately documented (IARC, 1972, 1974a,b, 1982, 1986; Bryant, 1987; Canada Environment Protection Act, 1997). Aniline has a limited documentation as an experimental carcinogen and an insufficient evidence as a human carcinogen (IARC, 1982).

It has also been known for a long time that tobacco smoke contains AA in conjunction with a series of carcinogenic compounds, such as polycyclic aromatic hydrocarbons and N-nitrosamines (Grimmer et al., 1987; Guerin et al., 1987; Canada Environment Protection Act, 1997). Epidemiological studies have suggested that AA are a relevant risk factor for the induction of urinary bladder cancer in smokers (Tannenbaum, 1990; Meerman and Van De Poll, 1994; Morabia et al., 1996;

* Corresponding author. Tel.: +39-055-4271323.
E-mail address: dolara@ds.unifi.it (P. Dolara).

0045-6535/01/$ - see front matter
PII: S0045-6535(00)00109-0

Vineis, 1981a,b; Vineis and Terracini, 1990). Since haemoglobin adducts of some AA have been detected in non-smokers, the possibility exists that indoor exposure to AA from passive smoke and outdoor AA contamination may have some toxicological effects (Bryant et al., 1987; Ronco and Vineis, 1990; Pieraccini et al., 1992).

AA originate in tobacco smoke from the pyrolysis of amino acids and are more abundant in side-stream than in main-stream smoke (Patrianakos and Hoffman, 1979; Hoffman and Winder, 1971, 1986; Neumann et al., 1990). Although, some AA are present as contaminants of food and water (Kiese, 1974; IARC 1986), most of them are absorbed through the respiratory route and then activated to N-hydroxylamine intermediates. N-hydroxylamines, excreted by the kidney, generate arylnitrenium ions, powerful electrophylic compounds, which bind the DNA of bladder epithelial cells and initiate carcinogenesis (Guerin, 1987; Hoffmann and Winder, 1971, 1986; Kadlubar, 1990; Del Santo et al., 1991; Matanosky et al., 1995).

We previously developed an original method for measuring AA in air samples, which enabled us to measure minute concentrations of AA with gas chromatography–mass spectrometry (GC/MS) (Pieraccini et al., 1992). Given the existing debate about the toxicological relevance of environmental tobacco smoke, we thought it of interest to measure the levels of selected AA in homes and non-domestic environments with variable contamination from tobacco smoke and in outside air. Our goal was to determine the concentration of AA to which people are exposed by breathing contaminated air. To accomplish this goal we selected 9 homes and 22 non-domestic environments in Florence, Italy, representing the most common sites in which non-smokers are exposed to environmental tobacco smoke. We also selected a series of outdoor locations in Italy, with variable population size and industrial development and we determined 10 AA in different periods of the year, to establish the level of air contamination.

2. Experimental

2.1. Chemicals

Pentafluoropropionic anhydride (PFPA) for derivatization was purchased from Fluka Chemie AG (Buchs, Switzerland); trimethylamine hydrochloride (TMA) from Aldrich Chemicals (Milwaukee, WI, USA). Sodium sulphate anhydrous, sodium hydroxide pellets and 0.3 nm molecular sieves were purchased from Merck (Darmstadt, Germany). All solvents were Merck analytical grade; solvents and the other materials were periodically tested, to confirm the absence of interfering substances. $^{2}H_5$-Aniline was purchased from Aldrich Chemical (Milwaukee, WI, USA) and ^{13}C-2-toluidine from Cambridge Isotope Laboratory (Baumgarten, Innerberg, Switzerland). 4-aminobiphenyl, $^{2}H_9$-4-aminobiphenyl and 3-amino-biphenyl were kindly supplied by Professor Tannenbaum (M.I.T., Cambridge, MA, USA). We obtained 2-naphthylamine from Sigma, Milan, Italy. Aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, 2,3-dimethylaniline, 2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline were obtained from Fluka Chemie AG (Buchs, Switzerland).

2.2. Sampling and sample preparation

Using two constant flow pumps, 1 m^3 of air was pumped into two apparatuses, each composed of three Drechsel bottles, containing 50 ml of 5% HCl. The two pumps were regulated at a rate of 33 ml/s and would sample 1 m^3 of air in about 8 h. An appropriate amount of an ethanol solution of the three internal standards ($^{2}H_5$-aniline, ^{13}C-2-toluidine and $^{2}H_9$-4-aminobiphenyl) was added to the first Drechsel bottle. AA were trapped in the acidic solutions as hydrochloric salts; the solutions were washed with diethyl-ether (150 ml), made alkaline up to pH 14 with sodium hydroxide and then extracted with *n*-hexane (3 × 150 ml). The solution of AA in hexane was made anhydrous with sodium sulphate, a saturated solution of TMA in *n*-hexane (40 μl) was later added and then derived with PFPA (25 μl), for 30 min at room temperature. The solution was then concentrated to a volume of 200 μl, at first using a rotary evaporator and finally under a gentle nitrogen stream. The sample was then ready for injection into the GC/MS system.

2.3. Analytical instrument

We used a GC/MS apparatus consisting of a Hewlett Packard (Palo Alto, CA, USA) gas-chromatograph model 5890 series II and of a mass spectrometer detector, model 5971-A. The system was equipped with an HP 7673 automatic injector and monitored by an HP G 1034B GC/MS Chemstation. Analytes were ionized by means of 70 eV electron impact and the generated ions were analysed by a quadrupole mass filter, with the application of selected ion monitoring (SIM), which improves sensitivity. We focused the spectrometer on the molecular ions $[M]^{+\bullet}$ of each PFP-derivative. The final solutions (1–2 μl) were injected in splitless mode (1 min purge off); the injector and transfer line were at 240°C and the temperature programme was: 50°C × 1 min/30°C per min up to 160°C/3°C per min up to 200°C/20°C per min up to 245°C/4.75 min at 245°C. The head pressure of helium (carrier gas) was 16 psi. We selected a capillary column with a Carbowax bound stationary phase (30 m, 0.25 mm i.d., 0.25 μm film thickness) purchased from Quadrex Corporation (New Haven, Connecticut, USA).

The precision and accuracy of the method were measured by repeating the same analytical procedure for four times after spiking the HCl solution contained in the first two bottles with known amounts of 17 AA and calculating the recovery of the analite after the procedure. The accuracy differed for different amines: aniline (104%), 2-toluidine (102%), 4-aminobiphenyl (88%). Precision (coefficient of variation) was: aniline (8%), 2-toluidine (9%), 4-aminobiphenyl (8%).

2.4. Sampling sites

(*A*) *Homes*. We measured AA in 4 homes of smokers and 5 homes of smokers.

(*B*) *Non-domestic sites*. We selected the following non-domestic environments (indicated in the figures with numerals: two hospital wards (1 and 9); a bank (2); a train compartment reserved for non-smokers (3); a public library (5); three offices (7, 8, 16); two hospital waiting rooms (9 and 15); a dentist office (10); three private clubs (11, 14 and 21); two newspaper offices (6 and 12); a middle school meeting room (13); a bar (17); a police office (18); a hairdresser lounge (19); a computer centre (20) and a discotheque (22). All these sites were located in the province of Florence, Italy. Each indoor site (in the Province of Florence, Italy) was monitored once. Each site was identified as an environment in which smoking was either allowed or not, according to what actually happened there, not according to the posted regulations, which are often not enforced in Italy.

Outdoor sampling locations. The determinations were carried out in the following locations: Florence and Siena, Livorno and Prato, industrial towns of Tuscany; Ponte a Elsa, Capannori and Santa Croce (small towns in Tuscany); Sticciano, rural village in Tuscany; Brindisi (a harbour in southern Italy) and in Taranto (an industrial town in southern Italy). Outdoor contamination measurements were repeated in Florence, Siena, Livorno and Brindisi, but in the other locations we made a single determination.

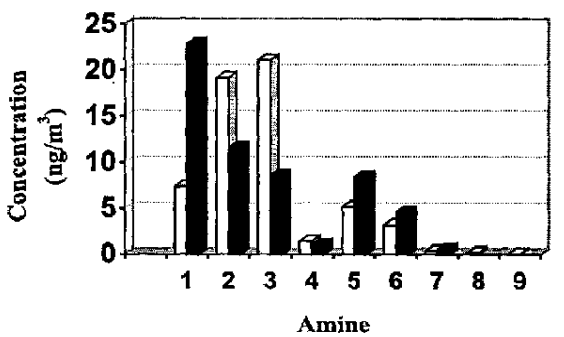

Fig. 1. Concentrations of AA in a sample of outdoor air (Brindisi centre, white columns) and in a sample of indoor air (office with smokers, black columns). Aniline values (129 and 259 ng/m³, respectively) were not included. (1) 2-toluidine, (2) 3-toluidine, (3) 4-toluidine, (4) 2,3-dimethylaniline, (5) 2,4-dimethylaniline, (6) 2,5-dimethylaniline, (7) 2,6-dimethylaniline, (8) 2-naphthylamine, (9) 4-aminobiphenyl.

3. Results

We measured the levels of all AA in each sampling. A typical pattern of AA in outdoor and indoor environments is shown in Fig. 1. Since the relative amounts of the different AA remained fairly constant, with the exception of aniline, to simplify the comparison of contamination between different sites, we expressed AA as sum of the levels of 9 AA (without considering aniline). The levels of AA in public buildings of the province of Florence, Italy, are shown in Fig. 2. Smoking environments are indicated with dark columns. The sum of 9 AA varied from a minimum of 3 ng/m³ in a hospital

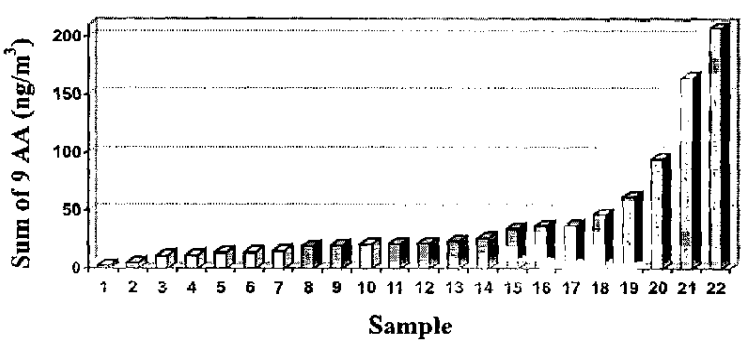

Fig. 2. Levels of 9 AA (excluding aniline) in the air of public buildings: 1: hospital ward I, 2: bank, 3: train compartment (no smoking), 4: hospital ward II, 5: public library, 6: newspaper office I, 7: office of non-smokers, 8: office with one smoker, 9: hospital waiting room I, 10: dentist office, 11: private club I, 12: newspaper office II, 13: school, 14: private club II, 15: hospital waiting room II, 16: office with two smokers, 17: bar, 18: police office, 19: hairdresser lounge, 20: computer centre, 21: private club III, 22: discotheque.

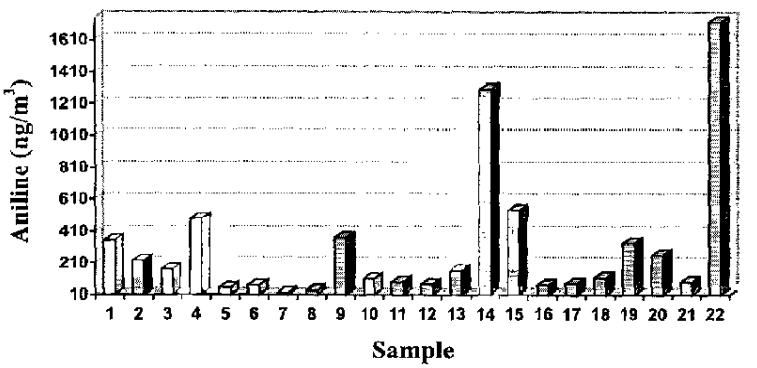

Fig. 3. Levels of aniline in the air of public buildings: 1: hospital ward I, 2: bank, 3: train compartment (no smoking), 4: hospital ward II, 5: public library, 6: newspaper office I, 7: non-smoking office, 8: smoking office, 9: hospital waiting room I, 10: dentist office, 11: private club I, 12: newspaper office II, 13: school, 14: private club II, 15: hospital waiting room II, 16: office with two smokers, 17: bar, 18: police office, 19: hairdreser lounge, 20: computer centre, 21: private club III, 22: discotheque.

ward to 207 ng/m^3 in a discotheque. Low levels (< 20 ng/m^3) were found in most non-smoking environments and moderately high levels (< 50 ng/m^3) in other locations in which smoking was allowed, but ventilation was adequate. A few sites had a very high contamination with AA (> 50 ng/m^3): a hairdresser, a computer centre, a private club and a discotheque. The situation was somewhat more confused considering aniline levels (Fig. 3). In fact, there was no significant correlation between the levels of aniline and of the level of the other 9 AA ($r = 0.364$) and no clear association with smoking. Actually, the highest values of aniline were recorded in hospital wards (351 and 483 ng/m^3).

The values of the sum of 9 AA in homes of smokers and non-smokers are shown in Fig. 4. The values recorded in homes of non-smokers were always below 20 ng/m^3 (range 5–11 ng/m^3) whereas homes of smokers, with one exception, had higher values (15–33 ng/m^3).

The sum of the 9 AA levels in the outside environment (Fig. 5) showed that some sites had very low contamination (< 20 ng/m^3). Among these, we found the rural site Sticciano and a non-industrial and relatively non-polluted city, like Siena. Some measurements indicated a very high air level of AA in Brindisi, a town which is next to a big petrochemical plant. Fig. 6 shows that the concentration of aniline in outdoor air was more erratic and not correlated to the other AA ($r = 0.263$); aniline was also high in a rural village like Sticciano (224 ng/m^3).

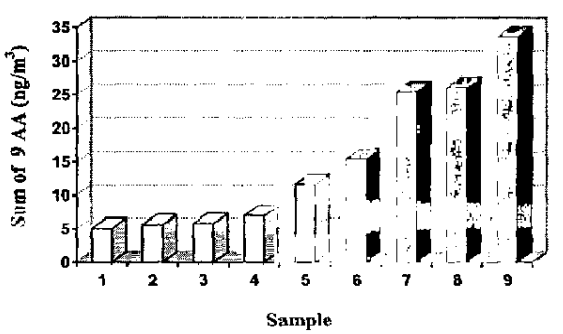

Fig. 4. Levels of 9 AA (excluding aniline) in air of the homes of non smokers (white columns) and of smokers (dark columns).

4. Discussion

The results described in this paper show that AA contamination is a widespread phenomenon in indoor as well as in outdoor environments. The source of aniline in indoor environment is likely due to the widespread presence of this amine in paints, cleaning fluids and house products, although no detailed analytical study has been published on this problem. It is harder to explain the presence of aniline in outdoor air, since we found it at relatively high concentration also in "clean" rural sites. However, the toxicological importance of aniline is unclear, since its role in human carcinogenesis is not firmly established and only a very high concentration can induce blood disorders such as metahemoglobinemia (Keise, 1974).

Our data show that very low levels of carcinogenic AA (< 20 ng/m^3) are present in indoor environments not contaminated by cigarette smoke. Such low levels are probably related to outdoor levels, but for practical reasons we could not obtain simultaneous measures of outdoor and indoor AA concentrations.

Among the indoor environments, several in which smoking is allowed had relatively high values of AA (20–50 ng/m^3) and some particularly high (over 100 ng/m^3).

In the outside air, the levels of the nine AA were generally low (< 20 ng/m^3). However, some measure-

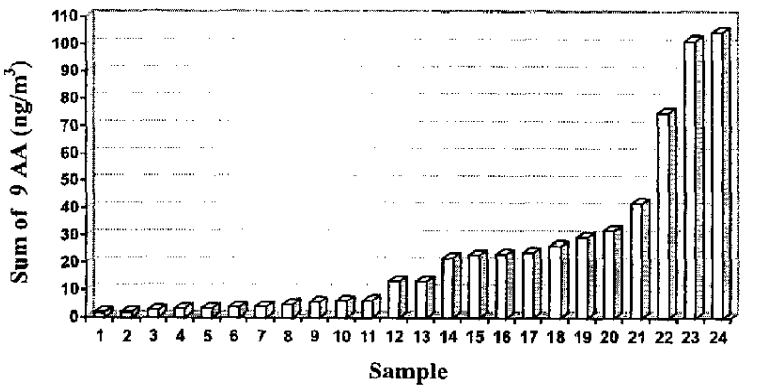

Fig. 5. Sum of 9 AA (excluding aniline) in the outdoor air of some Italian towns: 1: Ponte ad Elsa, 2: Florence outskirts I, 3: Florence outskirts II, 4: Siena I, 5: Sticciano, 6: Florence outskirts III, 7: S. Croce sull'Arno, 8: Siena II, 9: Taranto I, 10: Florence centre I, 11: Taranto II, 12: Leghorn (industrial zone) I, 13: Capannori, 14: Leghorn (industrial zone) II, 15: Leghorn (industrial zone) III, 16: Prato I, 17: Prato II, 18: Florence outskirts IV, 19: Florence outskirts V, 20: Brindisi centre I, 21: Brindisi centre II, 22: Brindisi centre III, 23: Brindisi (industrial zone) I, 24: Brindisi (industrial zone) II.

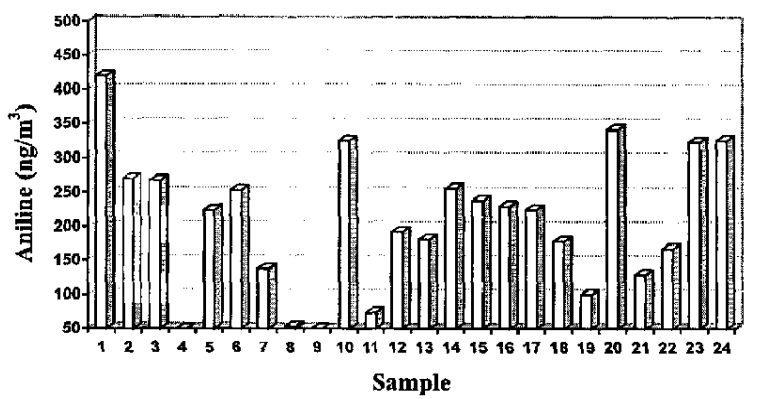

Fig. 6. Levels of aniline in the outdoor air of some Italian towns: 1: Ponte ad Elsa, 2: Florence outskirts I, 3: Florence outskirts II, 4: Siena I, 5: Sticciano, 6: Florence outskirts III, 7: S. Croce sull'Arno, 8: Siena II, 9: Taranto I, 10: Florence centre I, 11: Taranto II, 12: Leghorn (industrial zone) I, 13: Capannori, 14: Leghorn (industrial zone) II, 15: Leghorn (industrial zone) III, 16: Prato I, 17: Prato II, 18: Florence outskirts IV, 19: Florence outskirts V, 20: Brindisi centre I, 21: Brindisi centre II, 22: Brindisi centre III, 23: Brindisi (industrial zone) I, 24: Brindisi (industrial zone) II.

ments showed levels in the range measured in indoor smoking environments (i.e. outdoor determinations in Brindisi, an industrial town with a large chemical plant).

Our data might help in clarifying the source of indoor contamination of AA and its correlation with cigarette smoke. Whereas smoking habits in private homes should be a matter of free choice, the exposure to AA from passive smoke in public places cannot be controlled by individual preferences. To cope with this problem, some governments have issued strict regulations, forbidding smoking in many public places and work environments; some others have adopted less restrictive attitudes.

Our measurements show that respiratory exposure to AA varies considerably, ranging from very low (well-ventilated indoor environments and non-polluted cities), to moderately high (some indoor environments) to very high (discotheques, private clubs and some industrial towns).

The assessment of the toxicological implications of the presence of AA in air is difficult, given the lack of precise data of overall contamination. Passive smoking has been advocated as a risk factor for lung (Kurelec and Gupta, 1993) and breast cancer (Mommsen and Aagard, 1983). These epidemiological studies often focus on home exposure of non-smokers living with a smoking spouse. The exposure to AA due to contaminated air outside the home was never adequately evaluated, since no good data were available in the

literature. Therefore, no quantitative dose–response relationship was available for the evaluation of human cancer risk associated with exposure to AA. It is possible that high exposures to AA (such as spending a long time in a smoke contaminated environment or living next to a chemical plant) might have detrimental health effects (Woodward and McMichael, 1991; Zellweger, 1991).

It is more difficult to evaluate the effect of intermediate exposures, such as those of most outdoor locations, homes of smokers and some working environments. Most published studies have focuses on the effect of passive smoking at home, ignoring other sources of contamination. With a limited knowledge of the air levels of AA in different environments, the evaluation of the connection between exposure to AA from passive smoking and/or other sources becomes quite problematic.

Our data indicate that restriction of smoking in certain sites, proper ventilation in others, control of industrial emissions and reduction of the levels of AA in industrial products would be a reasonable strategy for reducing exposure to AA. Given the carcinogenic potency of AA, minimising human exposure with a multiplicity of approaches might be desirable.

Acknowledgements

This work was made possible by a grant of the Regional Government of Tuscany, by a grant of MURST-Italy and partially by a grant from Philip Morris. We are grateful to Dr. Nedo Mennuti of the "Comitato Empoli contro il fumo" and to Dr. G. Guerri for help in the location of sites and the collection of the samples; we thank A. Cerioli for the discussion of the experimental layout and of the manuscript.

References

Bryant, M.S., Skipper, P.L., Tannenbaum, S.R., Maclure, M., 1987. Hemoglobin adducts of 4-ABP in smokers and nonsmokers. Cancer Res. 47, 602–608.

Canada Environment Protection Act, 1997. Environment Canada's priority substances list assessment report. Database.

Del Santo, P., Moneti, G., Salvadori, M., Saltutti, G., Delle Rose, A., Dolara, P., 1991. Levels of the adducts of 4-ABP to hemoglobin in control subjects and bladder carcinoma patients. Cancer Lett. 60, 245–251.

Grimmer, G., Naujack, K.W., Dettbarn, G., 1987. Gaschromatographic analysis of polycyclic aromatic hydrocarbons, aza-arene, aromatic amines, in the particle and vapour phase of main stream and side stream smoke of cigarettes. Toxicol Lett. 35, 117–124.

Guerin, M.R., 1987. Formation and physicochemical nature of side stream smoke. IARC Sci. Publ. 81, 117–124.

Hoffman, D., Winder, E.L., 1971. A study of tobacco carcinogenesis. Tumor initiators, tumor accelerators, tumor promoting activity of condensate fraction. Cancer 27, 848–864.

Hoffman, D., Winder, E.L., 1986. Chemical constituents and bioactivity of tobacco smoke. IARC Sci. Publ. 74, 145–165.

Humphrey, W.G., Kadlubar, F.F., Guengerich, F.P., 1992. Mechanism of C8 alkylation of guanine residues by activated arylamines; evidence for initial adducts formation at N7 position. Proc.Natl. Acad. Sci. (USA) 89 (17) 8278–8282.

IARC, 1972. 4-aminobiphenyl, vol. 1.

IARC, 1974a. 2-naphtylamine, vol. 4.

IARC, 1974b. Aromatic amines and related compounds, vol. 7.

IARC, 1974c. Some aromatic azo compounds, vol. 8.

IARC, 1982. Some aromatic amines, anthraquinones and nitroso compounds and inorganic fluorides used in drinking water and dental preparations, vol. 27.

IARC, 1986. Tobacco smoking, vol. 36, pp. 189–226.

Kadlubar, F., 1990. Carcinogenic aromatic amine metabolism and DNA adducts detection in humans. Int. Symp. Princess Takamatsu Cancer Res. Fund 21, 329–338.

Kiese, M., 1974. Methenoglobinemia: A Comprehensive Treatise. CRC Press, Cleveland, Ohio, USA.

Kurelec, B., Gupta, R.C., 1993. Biomonitoring of aquatic systems. IARC. Sci. Publ. 124, 365–372.

Matanosky, G., Kanchanaraksa, S., Lantry, D., Chang, Y., 1995. Characteristics of nonsmoking women in NHANES I and NHANES II epidemiological follow up study with exposure to spouse who smoke. Am. J. Epidemiol. 142 (2), 149–157.

Meerman, J.H., Van De Poll, M.L., 1994. Metabolic activation routes of arylamines and their genotoxic effects. Environ. Health. Persp. 102 (Suppl. 6), 153–159.

Mommsen, S., Aagard, J., 1983. Tobacco as a risk factor in bladder cancer. Carcinogenesis (4), 335–338.

Morabia, A., Bernstein, M., Heritier, S., 1996. Smoking and breast cancer reconciling the epidemiologic evidence by accounting for passive smoking and or genetic susceptibility to tobacco smoke. Am. J. Epidemiol. 143 (9), 918–928.

Neumann, H.G., Hammerl, R, Hillesheim, W, Wildschutte, M., 1990. Role of genotoxic and nongenotoxic effects in multistage carcinogenicity of aromatic amines. Environ. Health. Persp. (88), 207–211.

Patrianakos, C., Hoffman, D., 1979. Chemical studies on tobacco smoke LXIV. On the analysis of aromatic amines in cigarette smoke.. J. Anal. Toxicol. 3, 150–154.

Pieraccini, G., Luceri, F., Moneti, G., 1992. A new gas chromatographic–mass spectrometric method for the analysis of primary aromatic amines in main and side-stream cigarette smoke. Rapid. Comm. Mass Spectrom. 6, 406–409.

Ronco, G., Vineis, P., 1990. Hemoglobin adducts formed by aromatic amines in smokers: sources of interindividual variability. British J. Cancer 61 (4), 534–537.

Tannenbaum, S.R., 1990. Hemoglobin carcinogen adducts as molecular biomarkers in epidemiology. Int. Symp. Princess Takamatsu Cancer Res. Fund. 21, 351–360.

Vineis, P., 1981a. Evidences of a multiplicative effect between cigarette smoking and occupational exposure in the etiology of bladder cancer. Cancer Lett. 14, 285–290.

Vineis, P., 1981b. Bladder cancer and smoking in males: types of cigarettes, age of start, effect of stopping, interaction with occupation. Int. J. Cancer 34, 165–170.

Vineis, P., Terracini, B., 1990. Biochemical epidemiology of bladder cancer. Epidemiology 1 (6), 448–452.

Woodward, A., McMichael, A.G., 1991. Passive smoking and cancer risk: the nature and uses of epidemiological evidence. Eur. J. Cancer 27 (11), 1472–1479.

Zellweger, J.P., 1991. Passive smoking. Schweiz Rundsch. Med. Prax. 80 (18), 492–495.